USE OF POLYPHENOLS EXTRACTED BY OLIVE MILL WASTEWATER AS DIETARY SUPPLEMENTS IN PIG: EFFECTS ON MEAT STORAGE AND QUALITY.

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Abstract: The oil industry, which is one of the leading agro-industrial sectors in the Mediterranean area and Campania Region, generates large amounts of olive mill wastewaters (OMWW) characterized by the presence of phenolic compounds which slow down its biodegradation. Among the various effects of polyphenols, in this note we would investigated their ability to preserve over time the nutritional quality of food. In detail, we reported the use of a phenolic extract obtained by the treatment of the OMWW to formulate a supplemented feed destined to Nero Casertana breed of pig, to evaluate the beneficial effects of phenolic compounds on meat quality and storage.

Keywords: *polyphenols, olive mill wastewater, tocopherol and malondialdehyde content.*

1. INTRODUCTION

Oxidative stress is considered the primary cause of numerous metabolic processes in pigs. Thus, the addition of antioxidants has been considered a preventive treatment.
Moreover, antioxidant supplementation provides an added value to the product obtained and can improve nutritional and functional food of meat.
Oxygen is a highly reactive atom that is capable of becoming part of potentially damaging molecules commonly called free radical or reactive oxygen species (ROS).
This antioxidant system includes serveral enzyme systems and numerous dietary antioxidant present in a wide variety of plant foods.
Recently, several experimental and epidemiological data have provided support to the traditional beliefs of the beneficial effect provided by olive derivates. In particular, the polyphenols present in olive leaves, olives, olive oil and olive mill wastewaters are potent antioxidant and radical scavengers with anti-inflammatory properties
The purpose of this work was to study the changes in the α-tocopherol and malondialdehyde content in Longissimus Dorsi of "Nero Casertana" pig muscle in order to evaluate the effects of phenolic compounds on the oxidative status during storage and aging of pig meat [1-2-3].
Several studies have focused on the potential use of antioxidants as natural supplements in animal nutrition, starting from the positive effects that this active molecules have in different species of animals and for the beneficial effects on human health .

In recent years studies have focused primarily on the supplementation of feed with polyphenols obtained from olive tree (Olea europaea L.) as they can reduce lipid oxidation and improve the nutritional and functional quality of meat, enhancing its stability.
The lipid oxidation is one of the major route for quality degradation in meat; the mechanistic approach involves generation of reactive oxygen species and formation of free radicals, which produce rancid odor, off-flavor

and surface discoloration of meat and meat products [4-5-6].
In this work the meat was storage both in fresh and preserved vacuum for 12 days.

In detail, the study proposed the following objectives:

- characterization of a phenolic extract obtained by the treatment of the oil mill wastewaters (OMWW);
- formulation and administration of a feed supplemented with the phenolic extract destined to pigs in the finishing phase;
- evaluation of the α-tocopherol content in the pig adipose tissue and muscle;
- evaluation of the malondialdehyde (MDA) content in pig meat, serum and liver.

2. MATERIAL AND METHODS

2.1 Experimental design

This study was carried out on 13 month old pigs (genetic type autochthonous Casertana) housed at a local farm (Mastrofrancesco Farm, Morcone, Benevento, Italy). Twenty-four animals were divided into two experimental groups (treated and control group). In the control group twelve animals of both sexes were fed with a standard diet for the finishing stage (wheat 20%, barley 20%, corn 20%, soy 10%; bran 20%; fava beans 10%). In the treated group twelve animals were fed with polyphenols extracted from olive mill wastewaters (OMWW) (4mg/Kg/day) added to the standard diet. Slaughter took place at 16 months of age at local slaughterhouses (Santa Croce del Sannio and Vitulano, Benevento, Italy).

The phenolic extract obtained from olive mill wastewater has been characterized in terms of phenolic content and antioxidant activity.

2.1 Characterization of phenolic compounds

To determine the composition of polyphenolic extract from OMWW the extract was dissolved in water and 25 µl was injected into the HPLC.
Phenols were identified by comparing the retention times and the absorption spectra with pure standards and with those reported in literature.

2.2 Determination of the antioxidant capacity

The determination of the antioxidant activity of the extract was based on ABTS radical cation scavenging activity assay.
The ABTS assay was applied using ABTS reagent (2,2-azinobis(3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt from Sigma Aldrich; the antioxidant activity was expressed in terms of mmoles of trolox equivalents per Kg of sample.

2.2 Determination of α-tocopherol content in meat

α-tocopherol content was evaluated during aging (12 days) both in fresh and preserved vacuum meat. Meat sampling was made at 0, 4, 8 and 12 days after the death of pigs.
First total lipid content was determined by Bligh and Dyer method using methanol-chloroform (2:1, v/v) [7].
Fat was dissolved in ethyl acetate and α-tocopherol content was determined by reversed-phase high-performance liquid chromatography (RP- HPLC using a LC-10ATvp liquid chromatograph) equipped with a SPD 10AVvp detector (Shimadzu, Japan).

The mobile phase was 1.2 mL/min flow methanol/water/acetonitrile (73,2:1,8:25 v/v/v). UV spectra for tocopherols were recorded at 290 nm, using alpha-tocopherol as external standard (r^2 > 0.99, five-point calibration curve).

2.2 Evaluation of lipid oxidation in meat

The evaluation of lipid oxidation in meat was based on the determination of malondialdehyde (MDA), a secondary lipid oxidation product. MDA, the compound used as an index of lipid oxidation, was determined by a specific kit (Sigma-Aldric) and quantified by spectrophotometric analysis.

Malondialdehyde content were evaluated during aging (12 days) both in fresh and preserved vacuum meat. Meat sampling was

made at 0, 4, 8 and 12 days after the death of pigs.

MDA content was also evaluated in serum and liver.

3. RESULTS

3.1 Phenolic content and antioxidant activity

The extract from olive mill wastewaters showed a good content in phenolic compounds.

The total phenolic content was of 97926 mg/ kg of sample (9.7% of polyphenols).

The composition of the extract phenolic powder used for experimentation is shown in the Table 1**.**

Table 1: Composition and antioxidant activity of the polyphenol extract from OMWW

TOTAL POLYPHENOLS (mg/kg)	94648
MAIN COMPOUNDS IDENTIFIED	
Hydroxytyrosol (mg/kg)	20829
Hydroxytyrosol derivative 1 (mg/kg)	3192
Hydroxytyrosol derivative 2 (mg/kg)	8738
Tyrosol (mg/kg)	3947
Caffeic acid or derivative (mg/kg)	9991
Verbascoside or caffeic acid derived (mg/kg)	17449
TOTAL ANTIOXIDANT ACTIVITY (mmoli trolox/Kg of sample)	8521

The main compounds contained in the extract from OMWW were represented by hydroxytyrosol and its derivated, verbascoside and caffeic acid (Table 1).

According to literature the ortho-diphenols like hydroxytyrosol and caffeic acid are the most powerful antioxidants [8-9].

Do to the presence of phenolic antioxidants, OMWW's extract showed a significant antioxidant activity on the basis of ABTS assay. The antioxidant activity was estimated at 8521 mmoles of trolox equivalents per Kg of sample.

3.2 α-tocopherol content

The α-tocopherol was identified by HPLC analysis, both in muscle than in the adipose tissue of control and treated pig (Table 2).

α-tocopherol content was higher in the adipose tissue than in the muscle both for the two groups.

The presence of tocopherols is only related to the vitamin E taken from pigs through the diet in the semi-wild breeding.

Table 2: α-tocopherol content in the meat and adipose tissue of the control and treated pigs during fresh and vacuum packed storage.

FRESH					
Pigs	**Sample**	**Tocopherols (mg a-tocoferolo/100 g, fresh weight)**			
		average		**sd**	
CONTROL GROUP	**Muscle**	0,06	±	0,01	
	Adipose tissue	0,22	±	0,02	
TREATED GROUP	**Muscle**	0,05	±	0,01	
	Adipose tissue	0,20	±	0,01	

UNDER VACUUM					
Pigs	**Sample**	**Tocopherols (mg a-tocoferolo/100 g, fresh weight)**			
		average		**sd**	
CONTROL GROUP	**Muscle**	0,07	±	0,03	
	Adipose tissue	0,22	±	0,02	
TREATED GROUP	**Muscle**	0,08	±	0,01	
	Adipose tissue	0,23	±	0,02	

3.3 Malondialdehyde content

The susceptibility of raw *Longissimus dorsi* muscle to lipid oxidation during aging as a function of dietary supplementation with polyphenols obtained from olive mill wastewaters is illustrated in Table 3.

Longissimus dorsi MDA level was reduced in the meat samples of treated group compared

with control group (Table 3). These differences in MDA levels resulted more evident in the samples collected at 8 and 12 days of aging stored in vacuum packed, that notoriously favorite the storage of food.

Table 3: *Longissimus dorsi* MDA contents of treated and control pig, collected at 0,4,8,12 days of aging and storage at 0/-2°C fresh and vacuum packed.

		LONGISSIMUS DORSI			
		CONTROL GROUP		TREATED GROUP	
		Fresh	Under vacuum	Fresh	Under vacuum
		MDA (nmol/microgrammo of muscle)			
Aging	0	0,40	0,40	0,376	0,376
	4 days	0,836	0,664	0,429	0,374
	8 days	0,904	0,690	0,646	0,398
	12 days	0,942	0,914	0,686	0,629

In the similar manner, also, in the serum and liver, MDA levels were reduced in the treated pig compared with control group (table 4).

The inhibition of lipid oxidation by OMWW is probably the result of various phenolic compounds with antioxidant activity that entered the circulatory system and then were taken up and stored in pork muscle. However, the bioavailability of these active constituents could not be directly demonstrated due to lack of adequate analytical methodology and equipment.

Anyway, a few recent *in vivo* studies have demonstrated the inhibitory potential of feed supplementation with olive leaves on lipid oxidation of turkey breast fillets and of raw *Longissimus Dorsi* muscle of pig during refrigerated storage [1-10-11].

Table 4: serum and liver MDA contents in the treated and control pigs.

SERUM

TREATED GRIOUP		CONTROL GROUP	
MDA (nmol/µl of serum)			
average	*SD*	*average*	*SD*
0,04	± 0,02	**0,08**	± 0,01

LIVER

TREATED GRIOUP		CONTROL GROUP	
MDA (nmol/µg of tissue)			
average	*SD*	*average*	*SD*
1,04	± 0,03	**1,64**	± 0,04

4. CONCLUSIONS

Our results suggest that enrichment the use of polyphenols obtained from OMWW as feeding supplement to the standard diet of pigs reduce lipid oxidation in muscle and adipose tissue in both types of storage, fresh and preserved vaccum.

So, thanks to the antioxidant effects of polyphenols that reduce lipids peroxidation it is possible improve meat quality and increase the time of shelf-life of the animal products.

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